

## CLAIMS

*what is claimed is:*

1. A method of estimating an amount of DNA in a cell, the method comprising:  
receiving an image of the cell, in which local values of a DNA image parameter  
correspond to amounts of DNA at the locations within the cell shown on the image; and  
estimating a total value of the DNA image parameter taken over at least a region of  
the cell where DNA is deemed to be present.
2. The method of claim 1, wherein the image of the cell is a digital representation of the  
cell.
3. The method of claim 1, wherein the cell is treated with an agent that selectively  
associates with DNA and emits a signal recorded as the DNA image parameter.
4. The method of claim 3, wherein the agent is a DNA stain.
5. The method of claim 1, wherein the DNA image parameter is a light or radiation  
intensity.
6. The method of claim 1, wherein the DNA image parameter is an electromagnetic  
radiation intensity provided at a particular wavelength or range of wavelengths.
7. The method of claim 1, wherein estimating the total value of the DNA image  
parameter comprises summing a per pixel value of the DNA image parameter over all pixels  
in the region of the cell where DNA is deemed to be present.
8. The method of claim 7, wherein the per pixel value of the DNA image parameter  
comprises a corrected per pixel intensity value.
9. The method of claim 8, wherein the corrected per pixel intensity value comprises the  
difference of an absolute pixel intensity value and an image background level.
10. The method of claim 9, wherein the corrected per pixel intensity value comprises the  
difference corrected for non-linearities in an image acquisition system used to produce the  
image of the cell.
11. The method of claim 1, further comprising converting the estimated total value of the  
DNA image parameter to an estimated value of the total DNA in the region where DNA is  
deemed to be present.
12. The method of claim 11, further comprising classifying the cell into a cell cycle state  
based on the estimated value of total DNA.
13. The method of claim 12, wherein classifying the cell into a cell cycle state comprises  
using a mixture model to operate on estimated values of total DNA for a population of cells.

14. The method of claim 1, wherein the region of the cell where DNA is deemed to be present is the cell nucleus.

15. A computer program product comprising a machine readable medium on which is provided program instructions for estimating an amount of DNA in a cell, the instructions comprising:

code for receiving an image of the cell, in which local values of a DNA image parameter correspond to amounts of DNA at the locations within the cell shown on the image; and

code for estimating a total value of the DNA image parameter taken over at least a region of the cell where DNA is deemed to be present.

16. The computer program product of claim 15, wherein the image of the cell is a digital representation of the cell.

17. The computer program product of claim 15, wherein the cell is treated with an agent that selectively associates with DNA and emits a signal recorded as the DNA image parameter.

18. The computer program product of claim 17, wherein the agent is a DNA stain.

19. The computer program product of claim 15, wherein the DNA image parameter is a light or radiation intensity.

20. The computer program product of claim 15, wherein the DNA image parameter is an electromagnetic radiation intensity provided at a particular wavelength or range of wavelengths.

21. The computer program product of claim 15, wherein estimating the total value of the DNA image parameter comprises summing a per pixel value of the DNA image parameter over all pixels in the region of the cell where DNA is deemed to be present.

22. The computer program product of claim 21, wherein the per pixel value of the DNA image parameter comprises a corrected per pixel intensity value.

23. The computer program product of claim 22, wherein the corrected per pixel intensity value comprises the difference of an absolute pixel intensity value and an image background level.

24. The computer program product of claim 23, wherein the corrected per pixel intensity value comprises the difference corrected for non-linearities in an image acquisition system used to produce the image of the cell.

25. The computer program product of claim 15, further comprising program instructions for converting the estimated total value of the DNA image parameter to an estimated value of the total DNA in the region where DNA is deemed to be present.

26. The computer program product of claim 25, further comprising classifying the cell into a cell cycle state based on the estimated value of total DNA.

27. The computer program product of claim 26, wherein classifying the cell into a cell cycle state comprises using a mixture model to operate on estimated values of total DNA for a population of cells.

28. The computer program product of claim 1, wherein the region of the cell where DNA is deemed to be present is the cell nucleus.

29. An image analysis apparatus for estimating the amount of DNA in a cell, the apparatus comprising:  
a memory or buffer adapted to store, at least temporarily, an image of the cell, in which image local values of a DNA image parameter correspond to amounts of DNA at the locations within the cell shown on the image; and  
a processor configured or designed to estimate a total value of the DNA image parameter taken over at least a region of the cell where DNA is deemed to be present.

30. The apparatus of claim 29, further comprising an interface adapted to receive the image of the cell.

31. The apparatus of claim 29, further comprising an image acquisition system that produces the image of the cell.

32. The apparatus of claim 29, wherein the cell is treated with an agent that selectively associates with DNA and emits a signal recorded as the DNA image parameter.

33. The apparatus of claim 29, wherein the DNA image parameter is a light or radiation intensity.

34. The apparatus of claim 29, wherein the processor estimates the total value of the DNA image parameter by summing a per pixel value of the DNA image parameter over all pixels in the region of the cell where DNA is deemed to be present.

35. The apparatus of claim 34, wherein the per pixel value of the DNA image parameter comprises a corrected per pixel intensity value.

36. The apparatus of claim 35, wherein the corrected per pixel intensity value comprises the difference of an absolute pixel intensity value and an image background level, which difference is corrected for non-linearities in an image acquisition system used to produce the image.

37. The apparatus of claim 29, wherein the processor also converts the estimated total value of the DNA image parameter to an estimated value of the total DNA in the cell.

38. A method of distinguishing between mitotic and interphase cells, the method comprising:  
receiving an image of a cell;

from the image, extracting values of one or more mitosis indicator parameters that correspond to a cell division state of the cell; and  
classifying the cell as either mitotic or interphase based upon the extracted values of the one or more mitosis indicator parameters.

39. The method of claim 38, wherein the image of the cell is a digital representation of the cell.

40. The method of claim 38, wherein the cell is treated with an agent that selectively associates with DNA and emits a signal recorded as a location of DNA within the cell.

41. The method of claim 38, wherein the signal is an electromagnetic radiation intensity.

42. The method of claim 38, wherein the agent is a DNA stain.

43. The method of claim 38, wherein the one or more mitosis indicator parameters include at least one of a variance in DNA concentration within the cell, the size of a region occupied by DNA within the cell, an average concentration of DNA within the cell, and a maximal concentration of DNA within the cell.

44. The method of claim 38, wherein the one or more mitosis indicator parameters include at least a variance in DNA concentration within the cell.

45. The method of claim 38, wherein classifying the cell as either mitotic or interphase comprises evaluating the one or more mitosis indicator parameters to determine a degree to which DNA within the cell has separated.

46. The method of claim 38, wherein classifying the cell as either mitotic or interphase comprises evaluating the one or more mitosis indicator parameters to determine a degree to which DNA within the cell has condensed into chromosomes.

47. The method of claim 38, wherein classifying the cell as either mitotic or interphase comprises evaluating the one or more mitosis indicator parameters to determine a degree to which DNA within the cell has concentrated into one or more discrete locations.

48. The method of claim 38, further comprising classifying a mitotic cell as pre or post-anaphase.

49. The method of claim 38, wherein classifying the cell as either mitotic or interphase comprises using a mixture model to operate on the values of the one or more mitosis indicator parameters for a population of cells.

50. The method of claim 38, wherein the image of the cell shows locations where the DNA exists within the cell.

51. A computer program product comprising a machine readable medium on which is provided instructions for distinguishing between mitotic and interphase cells, the instructions comprising:

code for receiving an image of a cell;  
code for extracting, from the image, values of one or more mitosis indicator  
parameters that correspond to a cell division state of the cell; and  
code for classifying the cell as either mitotic or interphase based upon the extracted  
5 values of the one or more mitosis indicator parameters.

52. The computer program product of claim 51, wherein the image of the cell is a digital  
representation of the cell.

10 53. The computer program product of claim 51, wherein the cell is treated with an agent  
that selectively associates with DNA and emits a signal recorded as a location of DNA  
within the cell.

15 54. The computer program product of claim 51, wherein the signal is an electromagnetic  
radiation intensity.

55. The computer program product of claim 51, wherein the agent is a DNA stain.

20 56. The computer program product of claim 51, wherein the one or more mitosis  
indicator parameters include at least one of a variance in DNA concentration within the cell,  
the size of a region occupied by DNA within the cell, an average concentration of DNA  
within the cell, and a maximal concentration of DNA within the cell.

25 57. The computer program product of claim 51, wherein the one or more mitosis  
indicator parameters include at least a variance in DNA concentration within the cell.

30 58. The computer program product of claim 51, wherein classifying the cell as either  
mitotic or interphase comprises evaluating the one or more mitosis indicator parameters to  
determine a degree to which DNA within the cell has separated.

59. The computer program product of claim 51, wherein classifying the cell as either  
mitotic or interphase comprises evaluating the one or more mitosis indicator parameters to  
determine a degree to which DNA within the cell has condensed into chromosomes.

35 60. The computer program product of claim 51, wherein classifying the cell as either  
mitotic or interphase comprises evaluating the one or more mitosis indicator parameters to  
determine a degree to which DNA within the cell has concentrated into one or more discrete  
locations.

40 61. The computer program product of claim 51, further comprising program instructions  
for classifying a mitotic cell as pre or post-anaphase.

45 62. The computer program product of claim 51, wherein classifying the cell as either  
mitotic or interphase comprises using a mixture model to operate on the values of the one or  
more mitosis indicator parameters for a population of cells.

63. The computer program product of claim 51, wherein the image of the cell shows  
locations where the DNA exists within the cell.

64. An image analysis apparatus for distinguishing between mitotic and interphase cells, the apparatus comprising:

a memory or buffer adapted to store, at least temporarily, an image of a cell; and  
a processor configured or designed to analyze said image in manner that extracts values of one or more mitosis indicator parameters that correspond to a cell division state and classifies the cell as either mitotic or interphase based upon the extracted values of the one or more mitosis indicator parameters.

65. The apparatus of claim 64, further comprising an interface adapted to receive the image of the cell.

66. The apparatus of claim 64, further comprising an image acquisition system that produces the image of the cell.

67. The apparatus of claim 64, wherein the cell is treated with an agent that selectively associates with DNA and emits a signal recorded as a location of DNA within the cell.

68. The apparatus of claim 64, wherein the one or more mitosis indicator parameters include at least one of a variance in DNA concentration within the cell, the size of a region occupied by DNA within the cell, an average concentration of DNA within the cell, and a maximal concentration of DNA within the cell.

69. The apparatus of claim 64, wherein the one or more mitosis indicator parameters include at least a variance in DNA concentration within the cell.

70. The apparatus of claim 64, wherein the processor classifies the cell as either mitotic or interphase by evaluating the one or more mitosis indicator parameters to determine a degree to which DNA within the cell has condensed into chromosomes.

71. The apparatus of claim 64, wherein the processor classifies the cell as either mitotic or interphase by evaluating the one or more mitosis indicator parameters to determine a degree to which DNA within the cell has concentrated into one or more discrete locations.

72. The apparatus of claim 64, wherein the image of the cell shows locations where the DNA exists within the cell.

73. A method of classifying a cell based on an image of that cell, the method comprising:  
receiving an image of a cell showing locations where nuclear DNA exists within the cell and also showing local amounts of the nuclear DNA;  
from the image, extracting values of one or more mitosis indicator parameters that correspond to a cell division state of the cell;  
from the image, estimating a total amount of DNA in the cell; and  
classifying the cell into a cell cycle phase based upon the one or more mitosis indicator parameters and total amount of DNA.

74. The method of claim 73, wherein the cell cycle phases into which the cell can be classified include G<sub>1</sub>, S, G<sub>2</sub>, and mitotic.

75. The method of claim 74, wherein the cell cycle phases into which the cell can be classified include G<sub>1</sub>, S, G<sub>2</sub>, pre-anaphase mitotic, and post-anaphase mitotic.

76. The method of claim 73, wherein classifying the cell comprises comparing at least one of the amount of DNA and the one or more mitosis indicator parameters to a model providing boundaries between certain cell cycle phases in parameter space.

77. The method of claim 76, further comprising generating the model from a population of cell images, for which the one or more mitosis indicator parameters have been extracted and the amount of DNA has been estimated.

78. The method of claim 77, wherein the model is generated using a mixture model.

79. The method of claim 77, wherein the population used to generate the model is an aggregation of cell images taken from multiple sources.

80. The method of claim 79, wherein the multiple sources are multiple wells on an assay plate.

81. The method of claim 77, wherein the population used to generate the model is a collection of images of control cells having a known distribution of cell cycle phases.

82. A computer program product of classifying a cell based on an image of that cell, the method comprising:

receiving an image of a cell showing locations where nuclear DNA exists within the cell and also showing local amounts of the nuclear DNA;

from the image, extracting values of one or more mitosis indicator parameters that correspond to a cell division state of the cell;

from the image, estimating a total amount of DNA in the cell; and

classifying the cell into a cell cycle phase based upon the one or more mitosis indicator parameters and total amount of DNA.

83. The computer program product of claim 82, wherein the cell cycle phases into which the cell can be classified include G<sub>1</sub>, S, G<sub>2</sub>, and mitotic.

84. The computer program product of claim 83, wherein the cell cycle phases into which the cell can be classified include G<sub>1</sub>, S, G<sub>2</sub>, pre-anaphase mitotic, and post-anaphase mitotic.

85. The computer program product of claim 82, wherein classifying the cell comprises comparing at least one of the amount of DNA and the one or more mitosis indicator parameters to a model providing boundaries between certain cell cycle phases in parameter space.

86. The computer program product of claim 85, further comprising program instructions for generating the model from a population of cell images, for which the one or more mitosis indicator parameters have been extracted and the amount of DNA has been estimated.

87. The computer program product of claim 86, wherein the model is generated using a mixture model.

88. The computer program product of claim 86, wherein the population used to generate the model is an aggregation of cell images taken from multiple sources.

89. The computer program product of claim 88, wherein the multiple sources are multiple wells on an assay plate.

90. The computer program product of claim 86, wherein the population used to generate the model is a collection of images of control cells having a known distribution of cell cycle phases.

91. An image analysis apparatus for distinguishing between mitotic and interphase cells, the apparatus comprising:

a memory or buffer adapted to store, at least temporarily, an image of a cell showing locations where nuclear DNA exists within the cell and also showing local amounts of the nuclear DNA; and

a processor configured or designed to (a) analyze said image in manner that extracts values of one or more mitosis indicator parameters that correspond to a cell division state, (b) estimate a total amount of DNA in the cell, and (c) classifying the cell into a cell cycle phase based upon the one or more mitosis indicator parameters and total amount of DNA.

92. The apparatus of claim 91, further comprising an interface adapted to receive the image of the cell.

93. The apparatus of claim 91, further comprising an image acquisition system that produces the image of the cell.

94. The apparatus of claim 91, wherein the cell is treated with an agent that selectively associates with DNA and emits a signal recorded as a location of DNA within the cell.

95. The apparatus of claim 91, wherein the one or more mitosis indicator parameters include at least one of a variance in DNA concentration within the cell, the size of a region occupied by DNA within the cell, and a maximal concentration of DNA within the cell.

96. The apparatus of claim 91, wherein the processor classifies the cell as either mitotic or interphase by evaluating the one or more mitosis indicator parameters to determine a degree to which DNA within the cell has condensed into chromosomes.

97. The apparatus of claim 91, wherein the processor estimates the total amount of the DNA in the cell by summing a per pixel value of a DNA image parameter over all pixels in the region of the cell where DNA is deemed to be present, wherein the DNA image parameter corresponds to amounts of DNA at the locations within the cell shown on the image.



98. The apparatus of claim 97, wherein the cell is treated with an agent that selectively associates with DNA and emits a signal recorded as the DNA image parameter.

99. The apparatus of claim 97, wherein the per pixel value of the DNA image parameter comprises a corrected per pixel intensity value.

100. The apparatus of claim 99, wherein the corrected per pixel intensity value comprises the difference of an absolute pixel intensity value and an image background level.

101. A computer assisted method of determining parameters for classifying cells into stages of the cell cycle, the method comprising:  
obtaining cell images for a population of cells;  
organizing the cell images based upon at least one of (i) an amount of DNA in the cells and (ii) a mitotic discriminator for the cell images; and  
from the resulting organization of cell images, determining said parameters for classifying the cells into stages of the cell cycle.

102. The method of claim 101, wherein the population of cells includes cells treated under control conditions.

103. The method of claim 101, wherein the population of cells includes a first set of cells treated with a first concentration of a biologically active agent and a second set of cells treated with a second concentration of the biologically active agent.

104. The method of claim 103, wherein the population of cells further includes a third set of cells treated under control conditions.

105. The method of claim 101, wherein determining said parameters for classifying the cells into stages of the cell cycle comprises applying a mixture model to the organization of cell images.

106. The method of claim 105, wherein determining said parameters for classifying the cells into stages of the cell cycle comprises employing an expectation maximization fitting technique to the mixture model.

107. The method of claim 101, wherein said parameters for classifying the cells into stages of the cell cycle allow classification of cells into at least the following stages: G<sub>1</sub>, S, G<sub>2</sub>, and mitotic.

108. The method of claim 101, wherein said parameters for classifying the cells into stages of the cell cycle allow classification of cells into at least the following stages: G<sub>1</sub>, S, G<sub>2</sub>, and pre-anaphase mitotic and post-anaphase mitotic.

109. The method of claim 101, wherein the cell images are organized based upon both the amount of DNA in the cells and the mitotic discriminator for the cell images.

110. The method of claim 101, wherein the mitotic discriminator includes at least one of a variance in DNA concentration within the cell, the size of a region occupied by DNA within the cell, and a maximal concentration of DNA within the cell.

5 111. A computer program product comprising a machine readable medium on which is provided instructions for determining parameters for classifying cells into stages of the cell cycle, the instructions comprising:

code for obtaining cell images for a population of cells;  
code for organizing the cell images based upon at least one of (i) an amount of DNA  
10 in the cells and (ii) a mitotic discriminator for the cell images; and  
code for determining said parameters for classifying the cells into stages of the cell cycle based on the organization of cell images.

112. The computer program product of claim 111, wherein the population of cells includes  
15 cells treated under control conditions.

113. The computer program product of claim 111, wherein the population of cells includes  
a first set of cells treated with a first concentration of a biologically active agent and a  
second set of cells treated with a second concentration of the biologically active agent.  
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114. The computer program product of claim 113, wherein the population of cells further  
includes a third set of cells treated under control conditions.

115. The computer program product of claim 111, wherein the code for determining said  
25 parameters for classifying the cells into stages of the cell cycle comprises code for applying  
a mixture model to the organization of cell images.

116. The computer program product of claim 115, wherein the code for determining said  
30 parameters for classifying the cells into stages of the cell cycle comprises code for  
employing an expectation maximization fitting technique to the mixture model.

117. The computer program product of claim 111, wherein said parameters for classifying  
the cells into stages of the cell cycle allow classification of cells into at least the following  
stages: G<sub>1</sub>, S, G<sub>2</sub>, and mitotic.  
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118. The computer program product of claim 111, wherein said parameters for classifying  
the cells into stages of the cell cycle allow classification of cells into at least the following  
stages: G<sub>1</sub>, S, G<sub>2</sub>, and pre-anaphase mitotic and post-anaphase mitotic.

119. The computer program product of claim 111, wherein the cell images are organized  
40 based upon both the amount of DNA in the cells and the mitotic discriminator for the cell  
images.

120. The computer program product of claim 111, wherein the mitotic discriminator  
45 includes at least one of a variance in DNA concentration within the cell, the size of a region  
occupied by DNA within the cell, and a maximal concentration of DNA within the cell.